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VITELLOGENIN SYNTHESIS IN FEMALE LARVAE OF THE GYPSY MOTH, *LYMANTRIA DISPAR* (L.): SUPPRESSION BY JUVENILE HORMONE

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Abstract—1. Fat body from feeding-phase, last instar gypsy moth females incorporates L-[^{35}S]methionine *in vitro* into two vitellogenins with the same molecular masses (165 and 180 kDa) as the apo-vitellogenins found in the hemolymph and the apo-vitellins in the eggs.

2. Both apo-vitellogenins are observed in the medium of fat body cultures, but only the 180 kDa apo-vitellogenin is observed in extracts of cultured tissue.

3. Synthesis and accumulation of the apo-vitellogenins are suppressed in a dose-dependent manner by topical treatment with the juvenile hormone analog, methoprene, prior to day 4.

4. This suppression suggests that a declining juvenile hormone titre is involved in the initiation of vitellogenin synthesis.

INTRODUCTION

Vitellogenins are the hemolymph precursors of the insect yolk protein, vitellin. In most insects, the initiation of vitellogenin synthesis in the fat body and/or the presence of vitellogenin in the hemolymph of adult females first occurs about the time that yolk deposition begins (Keeley, 1985; Postlethwait and Giorgi, 1985; Vafopoulou-Mandalos and Laufer, 1987). Although the precise endocrine pathways which control vitellogenin synthesis differ among insects, the initiation and/or maintenance of the synthesis of vitellogenin by the fat body of adult females is stimulated, either directly or indirectly, by the presence of juvenile hormone (Hagedorn and Kunkel, 1979; Engelmann, 1983, 1984; Hagedorn, 1985; Keeley, 1985; Vafopoulou-Mandalos and Laufer, 1987; Kumaran, 1990).

In the Lepidoptera, differences exist among species with regard to the timing of vitellogenin synthesis during development and the role of juvenile hormone in regulating vitellogenin synthesis (Hagedorn and Kunkel, 1979; Postlethwait and Giorgi, 1985). For example, vitellogenin synthesis by the fat body of *Hyalophora cecropia* (L.) first occurs in female larvae at the time of larval–pupal apolysis, which is well before yolk deposition (Pan, 1971). Allatectomy of diapausing *H. cecropia* pupae had no influence on vitellogenin synthesis (Pan, 1977). The appearance of vitellogenin in immature *H. cecropia* pupae or pupal adult intermediates was not influenced by allatectomy of fourth or early fifth instar larvae (Pan, 1977). Injection of juvenile hormone I had no influence on vitellogenin synthesis in isolated diapausing abdomens of *H. cecropia* pupae (Pan, 1977). In contrast, neither vitellogenin synthesis nor yolk deposition in *Danaus plexippus* (L.) are detected until after adult emergence. Vitellogenin synthesis in *D. plexippus* was prevented by neck-ligation of newly emerged adults and restored by the injection of juvenile hormone I (Pan and Wyatt, 1976).

Mechanisms which regulate vitellogenin synthesis develop much earlier in females of the gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae), than in other insects. Apo-vitellogenin was detected in the hemolymph of 3-day-old, feeding-phase, last instar females (Davis *et al.*, 1990b; Kelly *et al.*, 1992). Using an ELISA method, Lamison *et al.* (1991) detected apo-vitellogenin in the hemolymph of 2-day-old last instars. The accumulation of apo-vitellogenin in the hemolymph was blocked by treatment of last instars with the juvenile hormone analog methoprene (Davis *et al.*, 1990a; Kelly *et al.*, 1992). Although vitellogenins have not been found naturally at such an early developmental stage in other insect species, juvenile hormone does suppress fat body synthesis of a number of non-vitellogenic, stage-specific proteins in at least four other lepidopteran larvae (Tojo *et al.*, 1981; Jones *et al.*, 1987; Ismail and Ray, 1988; Memmel and Kumaran, 1988). We report here on the role of juvenile hormone in regulating the *in vitro* synthesis of vitellogenin by the fat body of gypsy moth larvae and the *in vivo* accumulation of vitellogenin in the hemolymph.

MATERIALS AND METHODS

Insect rearing and chemical treatment

Larvae used in all experiments were derived from the New Jersey strain of *L. dispar* (O'Dell *et al.*, 1984). Handling of the eggs and preparation of the diet used to rear the post-embryonic stages were performed according to Bell *et al.* (1981). Post-embryonic stages were reared at 25 ± 1°C, 50–60% r.h. and on a photoperiodic regime of 16 hr light:8 hr darkness. Larvae were reared through the fourth stadium at a density of 10/180 ml cup. Larvae designated as day one of the last (5th) instar were selected 5 hr after lights-on following ecdysis during the previous lights-off

period. These larvae were reared at a density of 5/180 ml cup. Larvae designated as day −1 of the last instar were fourth instars in the second day of head capsule slippage. Female larvae were used in all experiments. Segregation of the sexes by size dimorphism was performed during the fourth instar when females are larger than males.

The juvenile hormone analogs, racemic 7-R,S-methoprene and juvenile hormone I (Calbiochem, San Diego, CA), and the anti-juvenile hormone, racemic fluoromevalonolactone (FMev; mol. wt = 148; D.C. Cerf, Zoecon-Sandoz, Palo Alto, CA), were dissolved in ethanol and topically applied (2 μl) to the dorsum of the abdomen. Although the day on which last instars were treated varied from −1 to 4 (5 hr after lights-on), all treated larvae were used for hemolymph collection and/or *in vitro* fat body culture on day 5 (5 hr after lights-on).

Hemolymph collection and tissue dissection

Larvae were anesthetized with CO_2 before hemolymph collection or tissue dissections were performed. Hemolymph was obtained by clipping a proleg and collecting 20 μl samples. Each sample was immediately diluted 1:6 with 4°C *Bombyx* saline (Okuda *et al.*, 1985) containing 0.1% phenylthiourea (to inhibit tyrosinases). Hemocytes were removed by centrifugation at 12,000 *g* and 4°C for 5 min. The supernatant was collected and stored at −90°C.

For tissue dissection, larvae were pinned ventral side down and the hemocoel was exposed by cutting the body wall along the entire dorsal midline. After removing the gut, the body cavity was rinsed with *Bombyx* saline. Perivisceral fat body tissue was then dissected from the hemocoel and washed for 5 min in *Bombyx* saline. Dissection was performed at room temperature (*ca* 22°C).

In vitro *culture of fat body*

Fat body was dissected and washed as described above. Excess saline was removed by blotting the tissue on filter paper. The tissue was then placed into pre-weighed (accuracy to ±0.01 mg) polypropylene tubes (1.5 ml, conical) containing 55 μl Grace's medium without L-methionine (Gibco®, Chagrin Falls, OH) which contained 1 μCi L-[^{35}S]methionine (1222 Ci/mmol, Amersham, Arlington Heights, IL) and unlabeled L-methionine where indicated. The tubes were weighed again to determine the wet weight of tissue (*ca* 10 mg) and the sealed tubes plus tissue were incubated for up to 12 hr at 26°C.

Following incubation, the culture was centrifuged at 12,000 *g* and 4°C for 10 min. As much of the incubation medium as possible was collected without disturbing the tissue pellet. To remove any remaining medium, the tissue was washed once with medium containing no L-[^{35}S]-methionine. This wash was discarded. Medium and tissue samples were stored separately at −90°C.

Extraction of protein from tissue

Previously frozen, cultured fat bodies were thawed on ice and extracted with 4°C *Bombyx* saline (100 μl final volume) which contained 10 mM ethylenediaminetetraacetic acid (EDTA), 1 mM phenylmethylsulfonyl fluoride and no $CaCl_2$. A plastic pestle that fit the incubation tube was used to homogenize the tissue. Homogenates were centrifuged at 12,000 *g* and 4°C for 20 min. The layer just above the pellet (middle layer) was designated as the tissue extract and stored at −90°C.

Determination of radioactivity incorporated into total protein

Aliquots (5 μl) of the media and tissue extracts were spotted onto discs of filter paper (1 cm diameter, Schleicher & Schuell No. 740-E) and allowed to air-dry (30 min). Protein was precipitated onto the discs according to the following method. Discs were soaked in 10% trichloroacetic acid (TCA) at 4°C for 10 min with gentle agitation, rinsed once with TCA at 4°C, then boiled for 10 min in TCA. Discs were then rinsed once with TCA at 4°C, soaked in TCA at 4°C for 10 min with gentle agitation, rinsed again with TCA at 4°C, rinsed once with ethanol at 4°C, air-dried for 30 min, and immersed in a liquid scintillation cocktail (ACS®, Amersham). Discs without sample were included as background controls. Aliquots (5 μl) of the media and tissue extracts were spotted on control discs which were used to determine radioactivity present before protein precipitation.

Statistical comparisons were made using a factorial design analyzed by the general linear models procedure of PC–SAS (SAS Institute, 1988). Probability (P) values in the Results section are for the independent type-III sums of squares F test. Student's t-test was used to determine if means were different from zero (Ho: $\mu = 0$).

Electrophoresis

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed according to Laemmli (1970). Separating gels (12 × 14 cm; 1.5 mm thick) were cast as a linear gradient of 7–15% acrylamide. Proteins in the gels were detected by staining with Coomassie Blue or by fluorography (Fluor-Hance®, Research Products International Corp., Mount Prospect, IL) enhanced autoradiography. The fluorographs were scanned using an Enhanced Laser Densitometer (LKB ULTROSCAN XL®). Preparation of partially purified egg vitellin was performed as described in Davis *et al.* (1990b).

RESULTS

Synthesis and release of protein by cultured fat body

Fat body from 5-day-old larvae incorporated the highest level of L-[^{35}S]methionine ($P \leq 0.002$) into

Table 1. Incorporation of L-[^{35}S]methionine into total protein by fat body from 5-day-old, female gypsy moth larvae incubated for 6 hr in different media

Modifications to Grace's medium		Incorporation* (cpm/mg tissue)	
Oxygen†	L-Methionine	Medium	Tissue extract
−	+	3392 ± 155a	5534 ± 203b
+	+	2067 ± 144c	4116 ± 138d
−	−	8459 ± 214e	12,756 ± 225f
+	−	5285 ± 202b	8619 ± 188e

*Values are means ± 95% confidence intervals and means followed by the same letter are not significantly different ($\partial = 0.05$; Tukey's w procedure; $N = 5$/modification).

†A plus sign indicates that O_2 was bubbled into the medium for 3 min and the air above the medium was evacuated with O_2 before the tube was caped and sealed with modelling clay. A dash indicates that nothing was done to the medium or tube (oxygen levels were atmospheric).

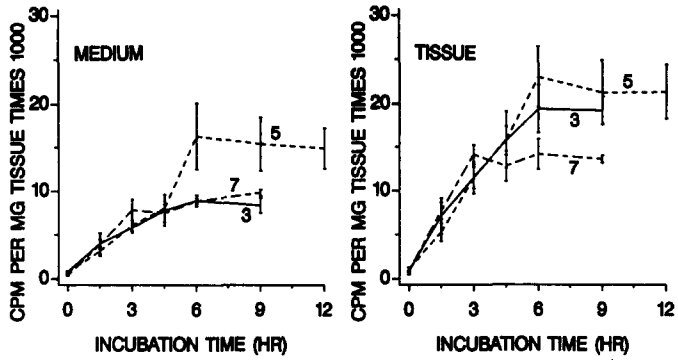

Fig. 1. Influence of incubation time and larval age on the *in vitro* incorporation of L-[^{35}S]methionine into the tissue (tissue extract) and medium (released) total protein by fat body from last instar, female gypsy moths. Numbers near each curve are larval age in days. Time 0 is fat body incubated for 5 min. Points represent means ±95% confidence intervals ($N = 3$ for days 3 and 7; $N = 5$ for day 5).

both tissue (tissue extract) total protein and medium (released) total protein when neither O_2 nor cold L-methionine were added to the incubation environment (Table 1). In each modification scheme, more label ($P \leqslant 0.02$) was present in tissue extract than in medium (Table 1).

In vitro incorporation of L-[^{35}S]methionine into tissue and medium total protein was maximum at 6 hr for fat body from either 3-day-, 5-day- or 7-day-old larvae (Fig. 1). The amount of L-[^{35}S]methionine incorporated into fat body total protein from larvae of all ages tested was greater ($P \leqslant 0.035$) than the amount released into the incubation medium. The amount of radioactivity in tissue and medium total protein for fat body incubated for 5 min (time 0 in Fig. 1) was not different from zero ($P > |t| \leqslant 0.001$).

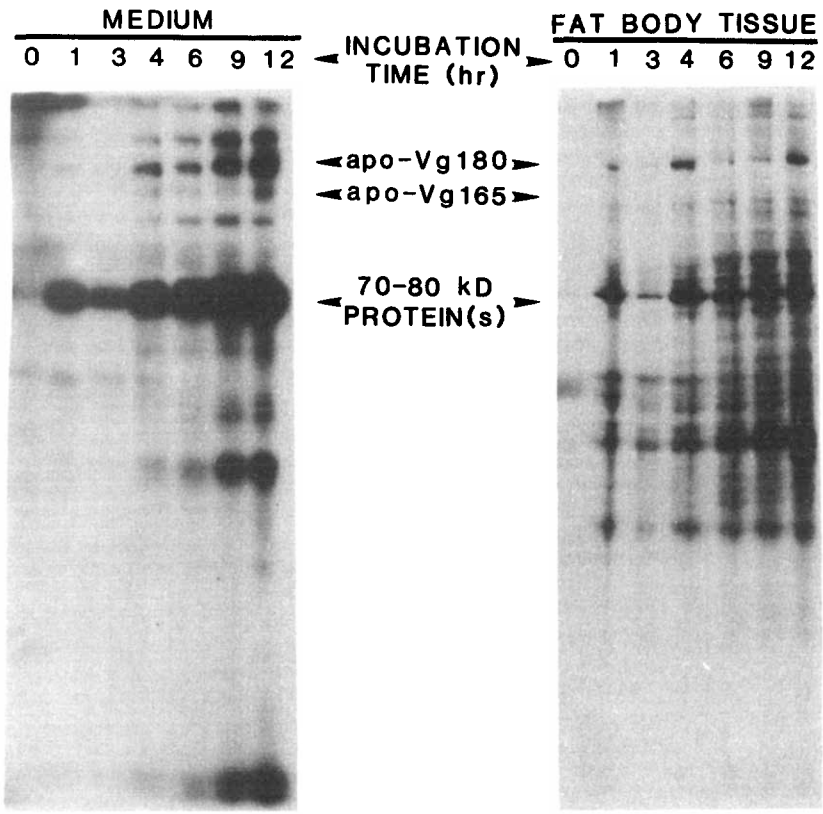

Fig. 2. Incorporation of L-[^{35}S]methionine into tissue (tissue extract) and medium (released) proteins by fat body incubated *in vitro* for up to 12 hr. Tissue was obtained from 5-day-old, last instar, female gypsy moths. Proteins were separated by SDS–PAGE and detected by fluorography enhanced autoradiography. Each lane contains 12,500 cpm of the total precipitated protein. Arrows indicate the positions of the 165 and 180 kDa apo-vitellogenins (apo-Vg) and presumed storage protein(s) (70–80 kDa proteins). These proteins were identified by comparing the fluorographs with the Coomassie stained position of these proteins in lanes loaded with egg vitellin or hemolymph from 5-day-old, last instar, females.

Maximum incorporation of L-[^{35}S]methionine into tissue and medium total protein was observed with fat body from 5-day-old, last instar females. Therefore, this larval age was used in all further experiments.

Fat body from 5-day-old, last instar, female larvae incorporated L-[^{35}S]methionine into the 165 and 180 kDa apo-vitellogenins and presumed 70–80 kDa storage protein(s) (Fig. 2). The apo-vitellogenins were identified by comparing the fluorographs with the Coomassie-stained position of bands in lanes of the same gel loaded with egg vitellin, which contained the 165 and 180 kDa apo-vitellins, or hemolymph from 5-day-old, last instar, females which contained the 165 and 180 kDa apo-vitellogenins and 70–80 kDa storage proteins (see the ETOH and egg vitellin lanes in Fig. 4a–e, for example). Densitometric scans of fluorographs, such as Fig. 2, showed that the incorporation of L-[^{35}S]methionine into the 165 and 180 kDa apo-vitellogenin band in the medium and the 180 kDa apo-vitellogenin band in the tissue increased with incubation time (Fig. 3). No 165 kDa apo-vitellogenin was observed in the tissue (Figs 2, 3), even though each lane of gels representing proteins in the medium or tissue was loaded with identical amounts of radioactivity. Accumulation of newly synthesized 165 kDa apo-vitellogenin in the medium increased with incubation time (Figs 2, 3), but was significantly ($P \leqslant 0.022$) less than the accumulation of newly synthesized 180 kDa apo-vitellogenin in the medium or tissue (Fig. 3). Incorporation of L-[^{35}S]methionine into the 70–80 kDa proteins also increased with incubation time, as did other proteins (Fig. 2). Tissue was incubated for 6 hr in all further experiments because detectable levels of the 165 kDa apo-vitellogenin were observed only in the medium after 4–6 hr of incubation (Figs 2, 3).

Influence of methoprene treatment

Methoprene was topically applied once for each age (days −1 to 4 of the last instar) at doses ranging from 1 to 1000 nmol (0.31–310 μg/larva). Measurements were performed on day 5, and data on hemolymph and fat body obtained from the same larva were analyzed with respect to day of treatment and dose applied.

Methoprene applied at 250 nmol or more on days −1, 1 or 2 blocked the accumulation of the 165 and 180 kDa apo-vitellogenins in the hemolymph by day 5 (Fig. 4a–c). These doses also blocked the incorporation of L-[^{35}S]methionine into the 165 and 180 kDa apo-vitellogenins in the medium and the 180 kDa apo-vitellogenin in the tissue extract of *in vitro* cultures of fat body from 5-day-old larvae (compare Fig. 5a–c with Fig. 6a–c).

Treatment of 3-day-old larvae with methoprene at 250 nmol or more suppressed, but did not block, the accumulation of the two apo-vitellogenins in the hemolymph by day 5 (Fig. 4d). These doses also suppressed the incorporation of L-[^{35}S]methionine into the two apo-vitellogenins in the medium and the 180 kDa apo-vitellogenin in the tissue extract when fat body from 5-day-old larvae was cultured *in vitro* (compare Fig. 5d with Fig. 6a–c).

Methoprene treatment of 4-day-old larvae did not appear to change the accumulation of the apo-vitellogenins in the hemolymph by day 5 (Fig. 4e), or the incorporation of L-[^{35}S]methionine into the apo-vitellogenins when fat body from 5-day-old larvae was cultured *in vitro* (compare Fig. 5e with Fig. 6a–c).

Doses of methoprene from 1 to 1000 nmol did not appear to alter the accumulation in hemolymph of the 70–80 kDa storage proteins (Fig. 4a–e; heavy band above the 66 kDa standard). These same doses did not influence the *in vitro* incorporation of L-[^{35}S]methionine into the storage proteins by fat body from 5-day-old larvae (Fig. 5a–e).

In the *in vitro* cultures of day 5 fat body obtained from treated larvae, L-[^{35}S]methionine-labeled 165 kDa apo-vitellogenin was present only in the medium. In addition, no 165 kDa apo-vitellogenin band was detected in the tissue lanes by densitometric scanning of the fluorographs. L-[^{35}S]-methionine-labeled 180 kDa apo-vitellogenin was present in both the tissue and medium (compare Fig. 5a–e with Fig. 6a–c). Moreover, the

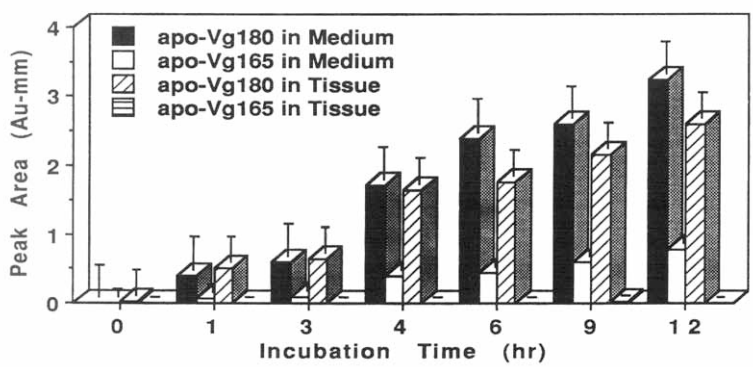

Fig. 3. Influence of incubation time on the relative incorporation of L-[^{35}S]methionine into the 165 and 180 kDa apo-vitellogenins (apo-Vg) in the tissue (tissue extract) and medium (released) proteins by fat body *in vitro*. Methods as in Fig. 2. Densitometric scanning of the fluorography enhanced autoradiographies represented by Fig. 2 was used to determine the relative amount of radiolabel (each lane contains 12,500 cpm of the total precipitated protein) in the 165 and 180 kDa apo-vitellogenin bands. Bars represent means ±95% confidence intervals for three 5-day-old, last instar, females. A different gel electrophoresis run was used for each larva.

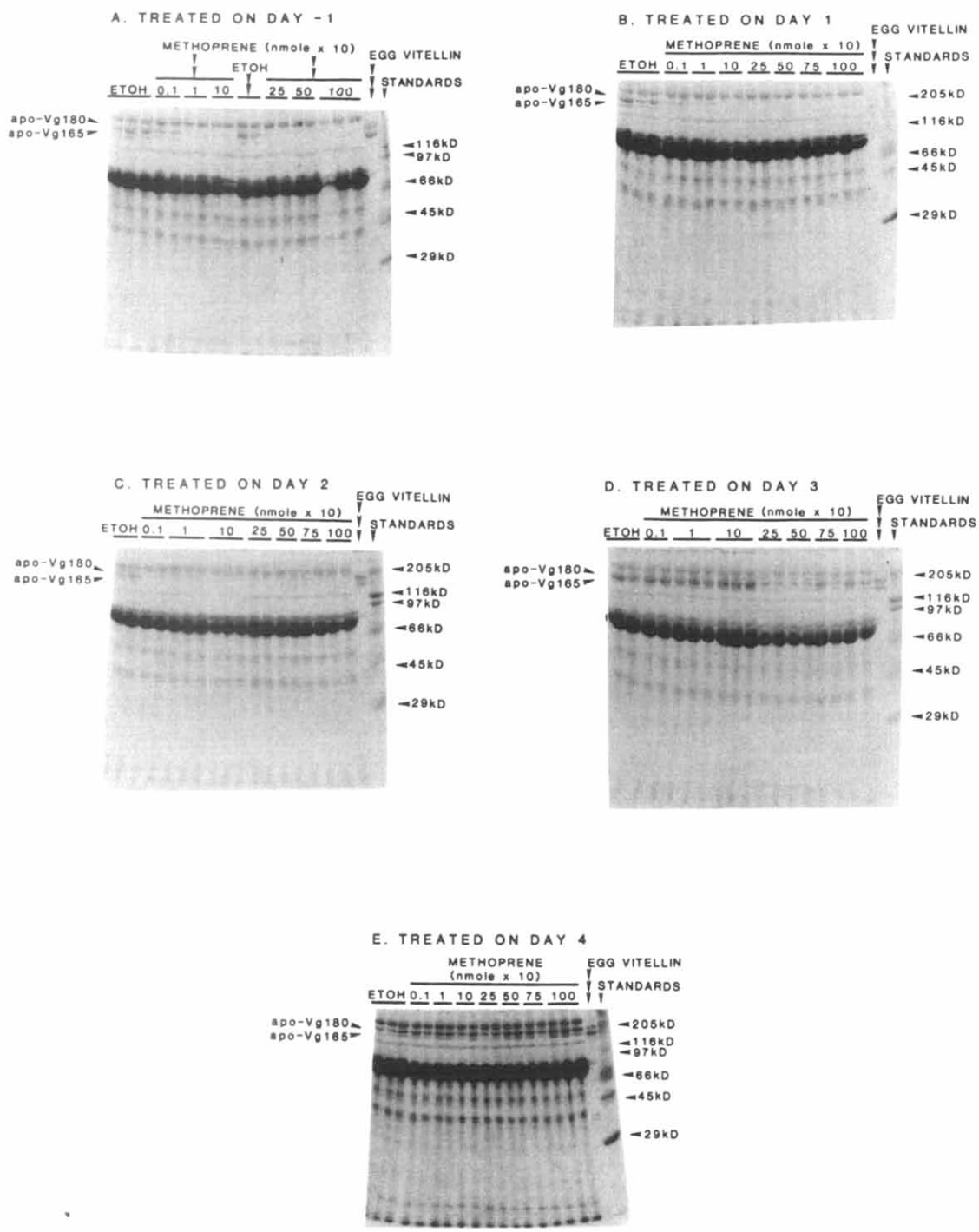

Fig. 4. Influence of methoprene, topically applied to different ages (indicated above each gel) of last instar, female gypsy moths, on the accumulation of protein in the hemolymph by day 5. Proteins were separated by SDS–PAGE and detected by Coomassie staining. Collected hemolymph was diluted 1:6 with *Bombyx* saline, and 5 μl of this dilution was loaded into each lane. Molecular weight standards (SDS-6H, Sigma Chemical Co., St Louis, Mo) are shown on the right and the positions of the 165 and 180 kDa apo-vitellogenins (apo-Vg) are shown on the left.

fluorographic densities of the L-[^{35}S]methionine-labeled 180 kDa apo-vitellogenin bands present in the medium were significantly ($P \leq 0.011$) greater than those of the labeled 165 kDa apo-vitellogenin bands present in the tissue or medium (Fig. 6a–c).

Influence of juvenile hormone I *and FMev treatment*

Treatment of 2-day-old last instars with 20 nmol or more of juvenile hormone I suppressed the accumulation of the apo-vitellogenins in the

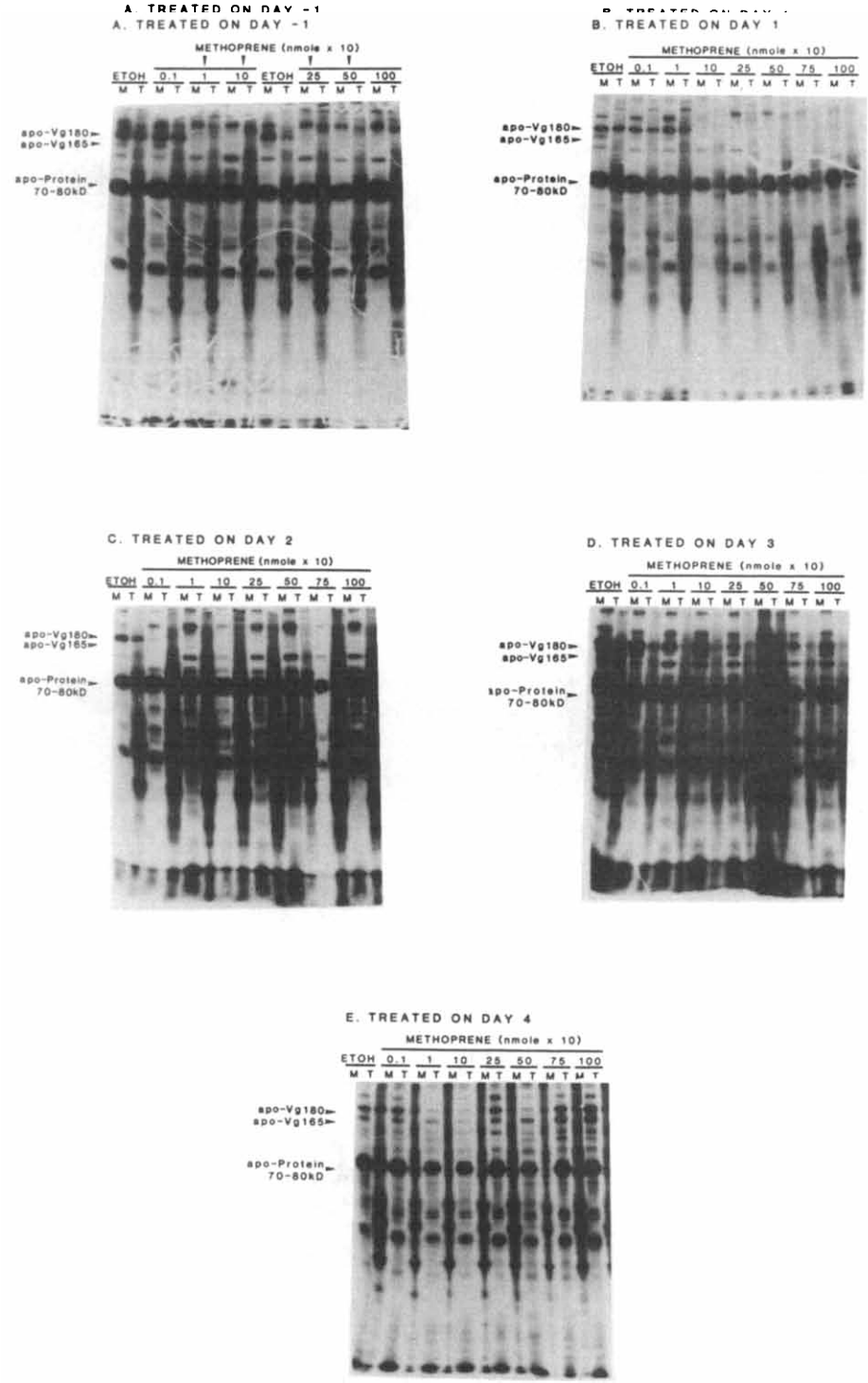

Fig. 5. Influence of methoprene, topically applied to different ages (indicated above each gel) of last instar, female gypsy moths, on the incorporation of L-[^{35}S]methionine into proteins present in the medium (M) and tissue (T) extract of *in vitro* cultures of fat body from day 5. The positions of the 165 and 180 kDa apo-vitellogenins (apo-Vg) and presumed storage proteins (apo-Protein 70–80 kDa) are shown on the left and identified as described in Fig. 2. Methods as in Fig. 2.

hemolymph by day 5 (Fig. 7). The doses used (0.01–200 nmol; 0.29–588 μg/larva) did not appear to alter the accumulation in the hemolymph of the non-apo-vitellogenin proteins (Fig. 7).

FMev was topically applied once to (−2)-day- and 1-day-old last instar larvae. Doses of 0.1–200 nmol (0.15–296 μg/larva) had no influence on accumulation of the 165 and 180 kDa apo-vitellogenins or

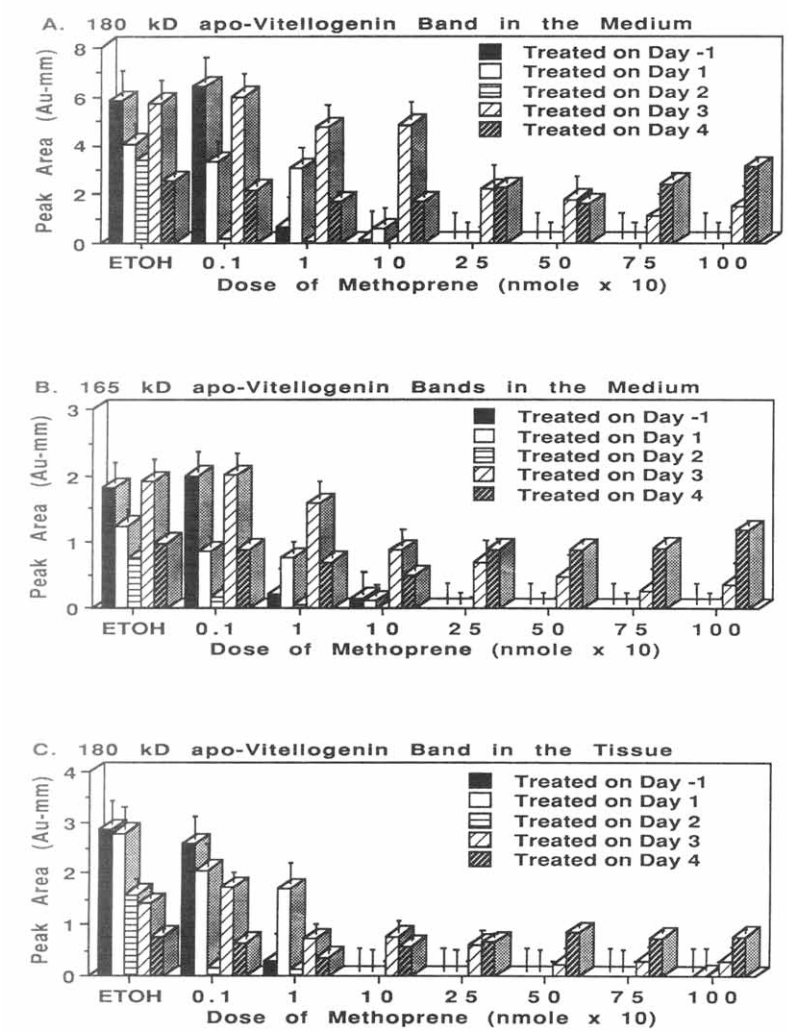

Fig. 6. Influence of the methoprene treatment described in Fig. 5 on the relative incorporation of L-[^{35}S]methionine into the 165 and 180 kDa apo-vitellogenin (apo-Vg) bands in the tissue (tissue extract) and medium (released) proteins from fat body incubations. Methods as in Fig. 2. Densitometric scanning of the fluorography enhanced autoradiographies represented by Fig. 5 was used to determine the relative amount of radiolabel (each lane contains 12,500 cpm of the total precipitated protein) in the 165 and 180 kDa apo-vitellogenin bands. Bars represent means ±95% confidence intervals for three larvae. A different gel electrophoresis run was used for each larva.

the non-apo-vitellogenins in the hemolymph by day 3 or 5. The FMev doses applied to 1-day-old larvae had no observable influence on development. All larvae treated with FMev on day-2 developed normally, except for 10% treated with 100 nmol and 60% treated with 200 nmol. These exceptions molted incompletely to the last instar and died.

DISCUSSION

The suppression of *in vitro* vitellogenin synthesis, by experimentally increasing the juvenile hormone level in female gypsy moth larvae prior to day 4 in the last instar, suggests that a low or declining hormone level is involved in the initiation of apo-vitellogenin synthesis during the first 3 days of the last instar. Once gypsy moth larvae begin apo-vitellogenin synthesis during days 2–3 in the last instar (Davis *et al.*, 1990b; Lamison *et al.*, 1991), methoprene treatment has a reduced influence on apo-vitellogenin synthesis.

These results demonstrate that synthesis of vitellogenin by the fat body of female gypsy moths differs from other insects in two important ways. First, vitellogenin synthesis was observed during the feeding-phase of the last instar. This is the earliest developmental stage of any insect species in which natural synthesis of vitellogenin has been documented. The synthesis of vitellogenin in a specific, pre-imaginal stage, however, is consistent with observations on other Lepidoptera which develop mature eggs before adult emergence (*H. cecropia*, Pan, 1971; *Manduca sexta* L., Sroka and Gilbert, 1971; *Bombyx mori* L., Kawaguchi and Doira, 1973; *Pieris rapae* L., Kim *et al.*, 1988). Second, elevated doses of juvenile hormone suppress vitellogenesis, suggesting that a decline in the juvenile hormone titre is

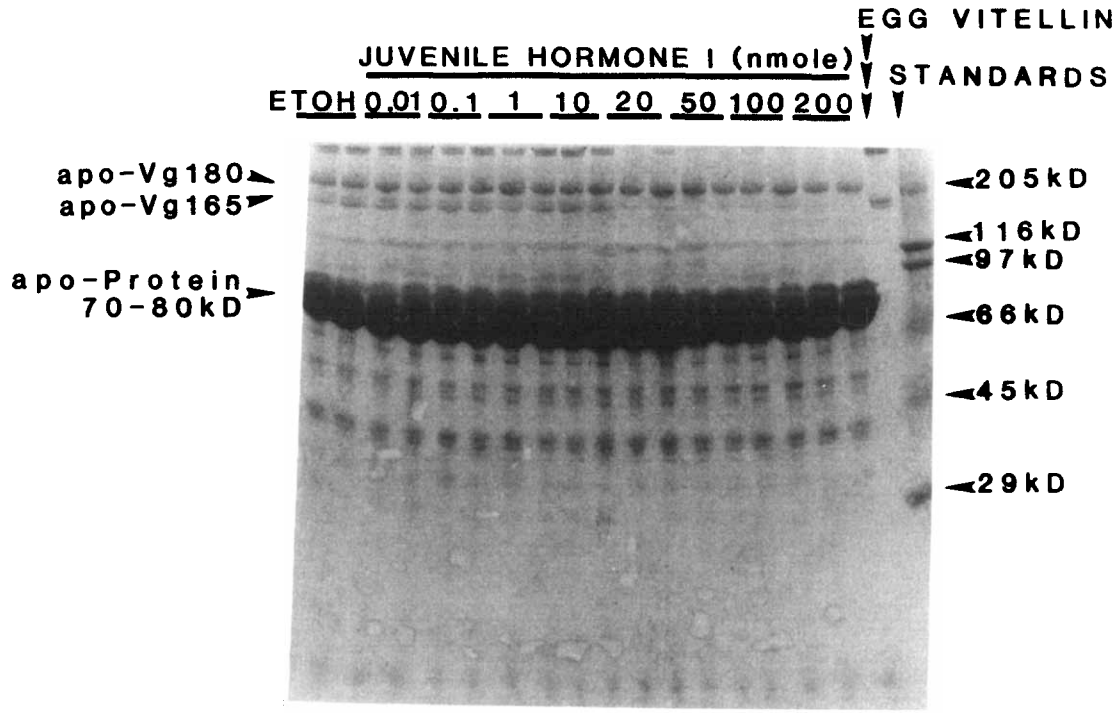

Fig. 7. Influence of juvenile hormone I, topically applied to 2-day-old last instar, female gypsy moth larvae, on the accumulation of protein in the hemolymph by day 5. Methods as in Fig. 4. Molecular weight standards (SDS-6H, Sigma Chemical Co.) are shown on the right and the positions of the 165 and 180 kDa apo-vitellogenins (apo-Vg) are shown on the left.

permissive for vitellogenin synthesis by the fat body of female gypsy moths. In contrast, vitellogenin synthesis by the fat body in most insect species which produce mature eggs as adults, occurs only when the level of juvenile hormone is increasing or already high (Hagedorn and Kunkel, 1979; Engelmann, 1983; Keeley, 1985; Vafopoulou-Mandalos and Laufer, 1987).

Methoprene and juvenile hormone I separately suppressed the accumulation of vitellogenin in the hemolymph (compare Figs 4c and 7), confirming that methoprene mimics the influence of the native hormone (Riddiford and Truman, 1978). Since topical application of FMev suppresses JH levels in *M. sexta* larvae (Baker *et al.*, 1986), one may have expected FMev treatment to rapidly lower the level of juvenile hormone in (−2)-day- or 1-day-old last instar gypsy moths and thereby cause an early (i.e. on day 3) or increased (i.e. on day 5) accumulation of apo-vitellogenin in the hemolymph. However, FMev had no influence on the accumulation of apo-vitellogenin in the hemolymph. When compared to the results of Baker *et al.* (1986) for *M. sexta*, approximately 12 times more FMev is needed to produce an influence on gypsy moth larval development. Lepidopteran species vary in their susceptibility to the influence of FMev on larval development (Quistad *et al.*, 1981; D. C. Cerf, personal communication), which suggests that FMev has little anti-juvenile hormone activity in gypsy moth larvae. Allatectomy may be the only alternative to FMev treatment, but allatectomy of "mature" ($\leqslant$2500 mg), last instar gypsy moths had no influence on the weight of ovaries in newly emerged adults (Wang and Yin, 1983). The influence of allatectomy during the late fourth or early last instars on reproductive processes in the gypsy moth has not been examined. Occurrence of precocious metamorphosis and early initiation of vitellogenesis in allatectomized larvae would suggest a close correlation between the initiation of vitellogenin synthesis and larval metamorphosis. The failure of methoprene treatment to block vitellogenin synthesis in 3- and 4-day-old last instar larvae suggests that, once initiated, vitellogenesis becomes refractory to methoprene treatment. Davis *et al.* (1990a) have examined the role, if any, of 20-hydroxyecdysone in the regulation of vitellogenin synthesis in gypsy moth larvae. They found 20-hydroxyecdysone to have no influence on vitellogenin synthesis in normal and methoprene-suppressed last instar females.

In a number of insect species, the responses of *in vitro* vitellogenin synthesis and accumulation to juvenile hormone treatment or allatectomy have been shown to be correlated with the *in vivo* responses to juvenile hormone treatment or allatectomy (Hagedorn and Kunkel, 1979; Engelmann, 1983; Postlethwait and Giorgi, 1985). In *L. dispar*, methoprene treatment suppresses both the synthesis of the apo-vitellogenins *in vitro* and in the *in vivo* accumulation of the apo-vitellogenins in the hemolymph. The correlation of these *in vitro* and the *in vivo* data suggest that apo-vitellogenin synthesis in last instar gypsy moths is permitted at low juvenile hormone titers.

Newly synthesized, 165 kDa apo-vitellogenin was present only in the medium of fat body cultures.

Therefore, this protein is either rapidly released from the fat body subsequent to synthesis, or final modifications to the 165 kDa form occur outside the fat body. Post-translational processing of larger apovitellogenin peptides has been observed in other insect species, but the precise biochemical pathways are not well known (Postlethwait and Giorgi, 1985; Vafopoulou-Mandalos and Laufer, 1987; Wojchowski *et al.*, 1986). Since the 180 kDa apovitellogenin was present in the medium prior to the appearance of the 165 kDa apo-vitellogenin, the 165 kDa form may be derived from the 180 kDa apo-vitellogenin.

In order to detect vitellogenin in the hemolymph or the medium and tissue extracts of fat body cultures, a relatively large amount of protein (*ca* 17 μg/lane) had to be loaded onto the gels. This resulted in the presence of a large amount of apo-protein in a band with a molecular weight ranging from 70 to 80 kDa. This molecular weight, synthesis by the fat body *in vitro*, and accumulation in the medium *in vitro*, and in the hemolymph *in vivo*, all suggest that these apoproteins are similar to the storage proteins detected in the hemolymph of other Lepidoptera (Levenbook, 1985). Although juvenile hormone regulates the synthesis of storage proteins by the fat body from some lepidopteran larvae (Tojo *et al.*, 1981; Jones *et al.*, 1987; Ismail and Ray, 1988; Memmel and Kumaran, 1988), only the vitellogenin, synthesized by fat body from the last instar, was found to be regulated by juvenile hormone in *L. dispar*.

In the last instar of gypsy moths (Jones and Yin, 1989; Tanaka *et al.*, 1989) and other Lepidoptera (Feyereisen, 1985; Riddiford, 1985; Bollenbacher, 1988), both the rate of *in vitro* biosynthesis of juvenile hormone by corpora allata and the titre of juvenile hormone in hemolymph peak early in the feeding-phase (day 1 of this instar for corpora allata activity in gypsy moths; Jones and Yin, 1989) and then decline over the course of the instar. Declines in both the synthesis of juvenile hormone by corpora allata and the titre of juvenile hormone in hemolymph are associated with the physiological events leading to larval–pupal metamorphosis (Riddiford, 1985; Bollenbacher, 1988). Our observations on juvenile hormone regulation of apo-vitellogenin synthesis correlate with those of Jones and Yin (1989) for corpora allata activity and those of Tanaka *et al.* (1989) for hemolymph juvenile hormone titre. It appears that the regulation of fat body vitellogenin synthesis in gypsy moth larvae is associated with endocrine events regulating larval–pupal metamorphosis.

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